NC STATE

Multiscale Modeling of Wound Healing

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

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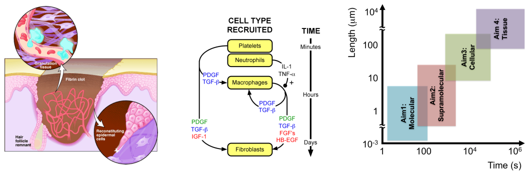
Wound healing is a multiscale phenomenon



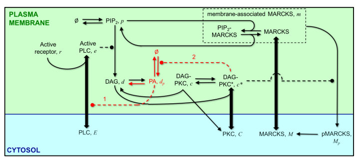
Modeling Approach

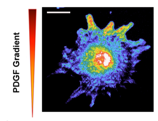
PDGF gradient → Aim 1 → Receptor activation, DAG patterns → Aim 2 → Myosin RLC pattern → Aim 3 → Fibroblast morphology, translocation → Aim 4 → Fibroblast invasion *in vivo*

Reaction-diffusion (PDEs) | Physico-chemical (stochastic) | Generalized random walks (stochastic) | Multi-agent (stochastic/hybrid)

A reaction-diffusion model to characterize amplification mechanisms in PDGF gradient sensing

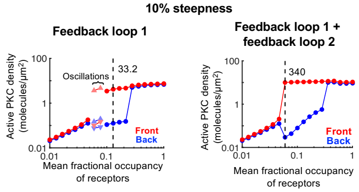
The phospholipase C (PLC)/protein kinase C (PKC) signaling pathway is required for chemotaxis of fibroblasts biased by a gradient of platelet-derived growth factor (PDGF), as during wound healing.



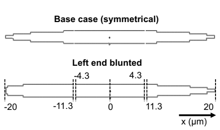


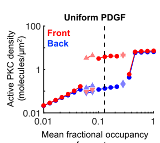
Chemotaxing cells maintain an internal gradient of diacylglycerol

To identify mechanisms capable of amplifying the sensitivity of this signaling pathway, reaction-diffusion models were formulated, and simulations show that inclusion of putative feedback loops at the level of lipid availability and metabolism yields a polarization circuit that is both sensitive and robust to varying gradient conditions.



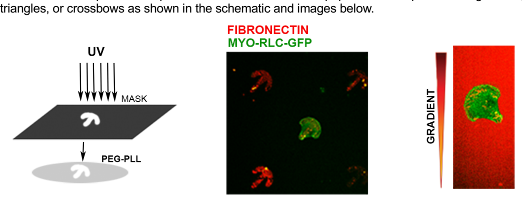
Subtle asymmetry in cell morphology causes spontaneous polarization of the proposed PLC/PKC network and influences external gradient sensing



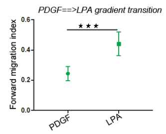


Steepness	Maximum front/back active PKC ratio: B → F	Maximum front/back active PKC ratio: F → B
0%	29.1	1 / 29.1
3%	30.7	1 / 27.6
10%	34.5	1 / 24.1
30%	47.0	38.0
67%	78.4	66.6

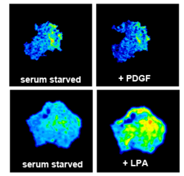
To address how the cell's geometry affects signaling and chemotaxis, experiments can be performed in which cells are plated onto patterns that force them to adopt particular shapes including circles, triangles, or crossbows as shown in the schematic and images below.



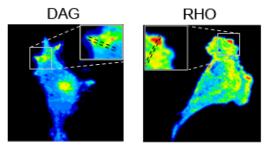
Diversity of chemotactic cues: LPA versus PDGF

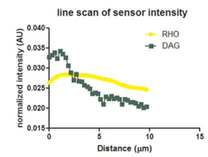


LPA is a potent chemoattractant that produces a robust directional response with FMI significantly higher than PDGF under serum free conditions.



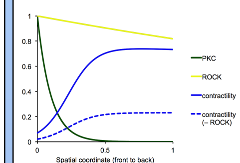
Using TIRF microscopy, cells expressing RBD-GFP were imaged upon uniform stimulation with PDGF and LPA independently. Analysis of fluorescent image intensity shows significant increase in RBD-GFP signal after LPA stimulation. However, PDGF stimulation did not produce an increase in RBD-GFP intensity providing verification of the utility of this probe.





LPA gradients prompt localized DAG production but global RHO activation in chemotaxing cells.

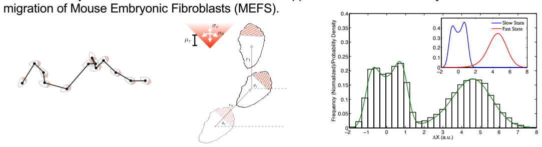
Mathematical model of Myosin regulation by PKC and ROCK in the context of chemotaxis



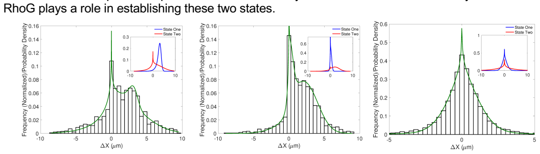
A key assumption of the model is that PKC activation by DAG is far more localized than ROCK activation by Rho-GTP; although both kinase activities are maximal at the (up-gradient) front of the cell, the relative gradient of ROCK activity is shallow, similar to that of receptor occupancy. The other key assumption is that phosphorylation of MLC on Ser1/2 shuts off myosin activity. Thus, the localized PKC activity trumps the high ROCK activity at the front, yielding a substantial gradient of myosin activity that increases from front to rear.

Stochastic methods for inferring states of cell migration

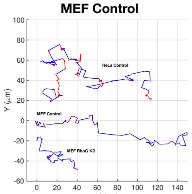
We developed computational tools, based on stochastic modeling, to analyze time series data for the position of randomly migrating cells. Our approach allows parameters that characterize cell movement to be efficiently estimated from time series data. We applied our methods to analyze the random migration of Mouse Embryonic Fibroblasts (MEFS).



Our analysis revealed that these cells exist in two distinct states of migration characterized by differences in cell speed and persistence. Further analysis revealed that the Rho-family GTPase RhoG plays a role in establishing these two states.



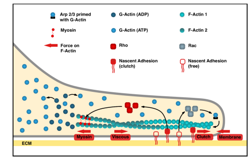
MEF Control | MEF RhoG KD | Hela Control

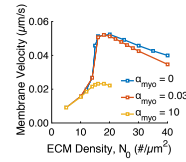


An important feature of our computational approach is that it provides a method for predicting the current migration state of an individual cell from time series data. Using this feature, we demonstrate that HeLa cells also exhibit two states of migration, and that these states correlate with differences in the spatial distribution of active Rac1.

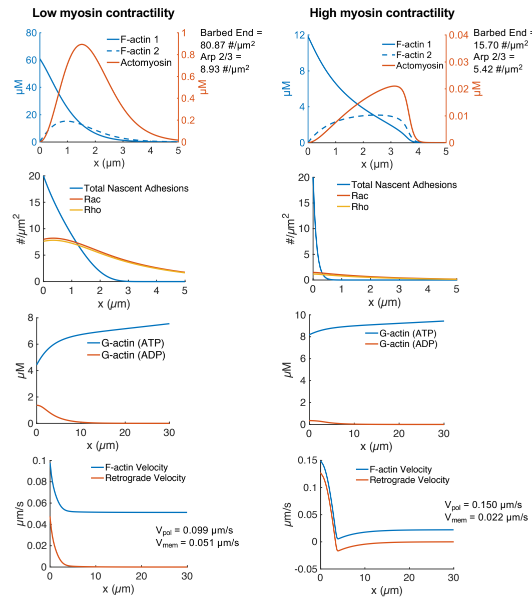
Integrative model of actin, adhesion, and signaling dynamics at the leading edge of migrating cells

A model of dendritic actin dynamics was combined with a physicochemical description of nascent adhesion assembly, which affects the F-actin network by resisting retrograde flow and activation of signaling pathways that activate the Arp2/3 complex at the cell's leading edge. In parallel, Rho/ROCK signaling is activated, leading to enhanced myosin II motor activity, which is also affected by the PLC/PKC pathway.





Protrusion (membrane) velocity as a function of the ECM density (N_0) is shown at different myosin contractility levels.

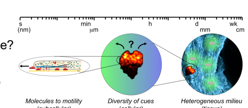


Our model predicts an optimal ECM (adhesion) density for maximal protrusion velocity. At lower ECM densities, not enough adhesions are formed, and most of the actin polymerization results in retrograde flow. At higher ECM densities, competition among increased barbed end density for G-actin and increased myosin activity reduce protrusion below optimum levels.

Future Challenges

Molecules to motility problem: how do we connect signaling and cytoskeletal dynamics to the mechanics of membrane protrusion/ retraction at the cell's leading edge?

Diversity of cues problem: PDGF is only one spatial cue for fibroblast migration, and hence it is paramount to consider the confluence of chemotactic, haptotactic, durotactic, and morphotactic cues.



Heterogeneous milieu problem: how do we integrate information about the spatial and biological heterogeneity of the wound, including macrophages secreting PDGF and the varying ECM densities?

References

- Asokan et al. (2014). Mesenchymal chemotaxis requires selective inactivation of myosin II at the leading edge via a noncanonical PLCγ/PKCα pathway. Dev. Cell, 31: 747-760.
- Mohan et al. (2017). A reaction-diffusion model explains amplification of the phospholipase C/ protein kinase C pathway in fibroblast chemotaxis. Biophys. J., 113: 185-194.
- Allen et al. (2018). Stochastic methods for inferring states of cell migration. bioRxiv, **doi:** https://doi.org/10.1101/249656.